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Transmembrane segment proteases

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Summary. Transmembrane segment proteases comprise a novel class of proteases that cleave substrates within hydrophobic membrane-spanning segments. They cleave in parts of proteins that upon first glance should be protected by the hydrophobic environment of the lipid bilayer. At present, no such protease has been isolated and biochemically characterized. They are defined according to the appearance of the respective cleavage products. All transmembrane segment proteases seem to participate in a regulated two-step proteolytic process that plays a central role in cellular regulation or is part of a protein degradation pathway.

Keywords: Endoplasmic reticulum; Signal peptide peptidase; γ-Secretase; SREBP.

Abbreviations: β-APP β-amyloid precursor protein; S1P site-1 protease; S2P site-2 protease; SPase signal peptidase; SPPase signal peptide peptidase; SREBP sterol regulatory element-binding protein; SCAP SREBP cleavage-activating protein.

Introduction

Proteases are found in almost every organelle of eukaryotic cells. They play constructive roles by proteolytic processing of precursor proteins or act in degradative processes. Wherever proteases hydrolyse peptide bonds, cleavage occurs in an aqueous compartment because the hydrophobic environment of the lipid bilayer precludes hydrolysis. Nevertheless, it has become clear that various organelles contain a new class of proteases that cleave substrate proteins within membrane-spanning segments. Such transmembrane segment proteases can be involved in the degradation of membrane proteins like the mAAA protease in the mitochondrial inner membrane (Leonhard et al. 1996) and may contribute to endoplasmic-reticulum (ER) degradation in addition to the proteasome (Jensen et al. 1995, Moriyama et al. 1998). Particular interest in transmembrane segment proteases arose from findings showing that some of these proteases generate products that regulate cell functions.

This review will emphasize the common principles of the new type of proteases and focus on the three transmembrane segment proteases that seem to be localized in the ER of mammalian cells. These proteases are (i) site-2 protease that activates sterol regulatory element-binding proteins, (ii) γ-secretase that is involved in the generation of amyloidogenic peptide Aβ, and (iii) signal peptide peptidase that releases signal peptide fragments.

Common features

All transmembrane segment proteases known at present act in a characteristic two-step proteolytic process. In a first step, the substrate protein is cleaved within its hydrophilic ectodomain. The residual membrane-bound fragment is next cleaved within the transmembrane segment and the resulting products are released into the cytosol or the exoplasmic space (Lyko et al. 1995, Bunnell et al. 1998, Duncan et al. 1998). The two-step process usually involves auxiliary factors that may activate the proteolytic cascade, regulate the proteases, or deliver the substrate.

According to the cleavage sites, transmembrane segment proteases can be devided in two groups (Table 1). Group 1 transmembrane segment proteases cleave close to the cytoplasmic end of membrane-spanning segments, in the region of the hydrophilic phospholipid head groups. Group 2 proteases cleave

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Table 1. Classes and substrates of transmembrane segment proteases[a]

Protease	Sequence of membrane-spanning segment and cleavage sites	Substrate protein	Intracellular localization
Group 1 (cleavage close to the cytosolic surface of the membrane)			
S2P	...DRSRILL**C**VLTFLCLSFNPLTSLLQWGGAH...	SREBP-2	endoplasmic reticulum (?)
nn[b]	...QLHLMYVAAAAFVLLFFVGCG**V**LLSRKR...	mNotch-1	plasma membrane (?)
Group 2 (cleavage in the centre of the transmembrane region)			
γ-Secretase[c]	...SNKGAIIGLMVGGVV**I**A**T**VIVITLVMLKKK...	β-APP	endoplasmic reticulum (?), endosomes (?)
SPPase[d]	...KGSRLLLLLV**VSN**LLLCQG...	signal sequence (bovine preprolactin)	endoplasmic reticulum
mAAA protease[e]	...HDNIMYYLVVILF**V**VGWILLSIIRNY...	COXII-DHFR	mitochondrial inner membrane

[a] In boldface, cleavage sites in the hydrophobic transmembrane segment; solid underline, exoplasmic side; broken underline, cytosolic side
[b] Notch proteins are ligand-activated transmembrane receptors in the plasma membrane. Cleavage by an unidentified protease in the transmembrane segment enables the released fragment to enter the nucleus (Schroeter et al. 1998)
[c] γ-Secretase(s) can cleave the transmembrane segment of β-APP at alternative sites, predominantly at the indicated positions, generating $A\beta_{40}$ (at I) or $A\beta_{42}$ (at T)
[d] SPPase cleaves the preprolactin signal peptide within the region of the small residues in the centre of the transmembrane segment. The exact cleavage site is not known (Lyko et al. 1995)
[e] mAAA protease is part of a proteolytic system for the ATP-dependent degradation of inner membrane proteins in mitochondria (Leonhard et al. 1996). COX II-DHFR, hybrid protein of cytochrome oxidase subunit II (1–74) and dihydrofolate reductase

in the centre of transmembrane segments. Actually, it is not known so far whether cleavage of a transmembrane segment occurs within the lipid bilayer. It is conceivable that the active site of group 1 proteases is placed within a groove that faces the cytosolic side of the membrane and is accessible to water for hydrolysis. Cleavage by group 2 proteases may require a more complex mechanism. Transfer of the substrate protein to either side of the membrane prior to cleavage seems unlikely since fragments can be released to both sides of the membrane. These proteases may be part of a multisubunit membrane protein complex similar to mitochondrial mAAA protease and provide water through the inside of the complex to the active site.

Site-2 protease: activation of a transcription factor

The best characterized transmembrane segment protease at present is site-2 protease (S2P) that cleaves sterol regulatory element-binding proteins (SREBPs) within one of their two membrane-spanning segments (Rawson et al. 1997). SREBPs are membrane-bound transcription factors that regulate the expression of proteins involved in synthesis and uptake of cholesterol and fatty acids in mammalian cells (Brown and Goldstein 1997). They share a common structure comprising three domains: (i) a cytosolic N-terminal domain containing a basic helix-loop-helix leucin zipper element and an acidic transcriptional activation sequence, (ii) a central segment with two membrane-spanning helices separated by a hydrophilic loop that is exposed toward the ER lumen, and (iii) a C-terminal regulatory domain that is exposed into the cytosol. SREBPs are anchored in the ER membrane and nuclear envelope and are released from the membrane in a two-step proteolytic process.

Processing of SREBPs is initiated by a protease, termed S1P, when sterols are depleted in a cell (Sakai et al. 1996). S1P cleaves SREBPs in the lumenal loop and generates two fragments, each anchored with a single membrane-spanning segment (Fig. 1a) (Sakai et al. 1998). Next, a second protease, S2P, cleaves the N-terminal intermediate at a leucine-cysteine bond that is located within the membrane-spanning segment (Duncan et al. 1998). This liberates the transcription factor domain that is released into the cytosol, enters the nucleus, and activates gene transcription.

Cleavage by S1P involves the regulatory protein SCAP (for SREBP cleavage-activating protein). This putative sterol sensor is a polytopic membrane protein that is localized in the ER membrane (Nohturfft et al. 1998). It consists of two domains: (i) an N-terminal domain with several membrane-spanning segments containing a putative sterol-sensing subdomain and (ii) a C-terminal cytosolic domain containing several WD repeats that bind to the regulatory domain of

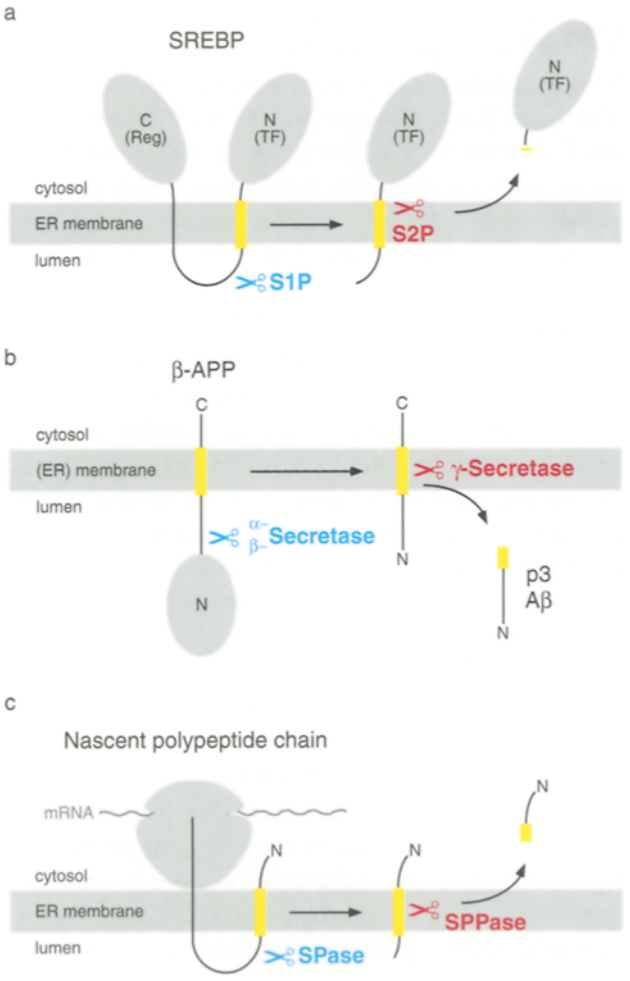

Fig. 1a–c. Transmembrane segment cleavage is part of a two-step process. **a** Processing of SREBP. S1P cleaves SREBP in the lumenal loop between the regulatory domain (*Reg*) and the transcription factor domain (*TF*). S2P next cleaves the N-terminal membrane-anchored intermediate within the membrane-spanning segment and liberates the transcription factor domain. **b** Proteolytic processing of β-APP. α- or β-secretase cleaves β-APP in its exoplasmic domain. The membrane-anchored fragment is next cleaved by γ-secretase within the transmembrane segment and peptide p3 or Aβ is released from the membrane. **c** Cleavage and processing of signal sequences. After membrane insertion, the signal sequence of a nascent polypeptide chain is cleaved off by SPase and further processed by SPPase. The N-terminal signal peptide fragment is released into the cytosol. Hydrophobic membrane-spanning segments are indicated in yellow

SREBPs. In sterol-depleted cells, SCAP and SREBP form a tight complex that recruits S1P and initiates the proteolytic processing of the bound SREBP (Sakai et al. 1997). The function of SCAP may be to deliver the substrate to S1P or vice versa and thereby to prevent the protease from unspecifically degrading other proteins in the ER lumen.

S1P and S2P have both been identified recently by complementation cloning. According to the deduced amino acid sequence, S1P is a C-terminally anchored type I membrane protein. Its large exoplasmic domain contains a catalytic triade that is typical for the subtilisin family of serine proteases (Sakai et al. 1996). Sensitivity toward endoglycosidase H suggests that S1P is N-glycosylated and localized in the ER. The analysis of the amino acid sequence of S2P revealed a HEXXH consensus sequence that is found in many families of metalloproteases (Rawson et al. 1997). Unlike any other known metalloprotease, S2P shows extensive hydrophobicity. It has up to five putative transmembrane segments, one of which containing the metalloprotease consensus sequence. The transmembrane segments may assemble to a substrate-binding groove that places the catalytic site into the membrane.

γ-Secretase: generation of amyloidogenic peptides

β-Amyloid precursor proteins (β-APPs) were the first proteins discovered that are processed within a transmembrane segment (Selkoe 1998). β-APPs are ubiquitously expressed type I membrane proteins with unknown function. During trafficking along the secretory pathway, they undergo endoproteolytic cleavages that yield amyloidogenic peptide Aβ and other products that are secreted. Aβ is the major constituent of amyloid plaques in the brain of patients with Alzheimer's disease (Iwatsubo et al. 1994).

Several proteolytic steps in β-APP processing have been described (Shoji et al. 1992). First, unidentified α- and β-secretase(s) cleave β-APP in its exoplasmic domain generating the C-terminally anchored fragments p3CT and A4CT, respectively (Fig. 1b). Both are then cleaved by unknown γ-secretase(s) within their transmembrane segment yielding nonamyloidogenic peptide p3 and amyloidogenic Aβ. Cleavage of A4CT occurs after residue 40, generating $A\beta_{40}$, occasionally after residue 42, generating $A\beta_{42}$. Increased levels of $A\beta_{42}$ might be sufficient to cause Alzheimer's disease.

Since α-, β-, and γ-secretases are not identified, their putative subcellular localization is derived from the appearance of the respective cleavage products. A portion of $A\beta_{40}$ seems to be generated in early endosomes following internalization of β-APP from the plasma membrane (Koo and Squazzo 1994). $A\beta_{40}$ and $A\beta_{42}$ can also be produced early in the secretory pathway. In neurons, for example, $A\beta_{42}$ is generated in

the ER whereas $A\beta_{40}$ is made in the trans-Golgi network (Hartmann et al. 1997, Lee et al. 1998). These observations suggest that pairs of secretases are present in several organelles. Secretases may be part of the protein degradation machineries in endosomes and the ER. Misfolded β-APP, for example, may fail the quality control in the ER and become degraded (Bunnell et al. 1998, Yang et al. 1998). After cleavages in the lumenal domain and the transmembrane segment, a portion of Aβ peptides may escape further degradation and may be secreted.

Generation of $A\beta_{42}$ in the ER is influenced by polytopic membrane proteins termed presenilins (De Strooper et al. 1997). Mutations in the genes encoding presenilin 1 and 2 account for the majority of cases of Alzheimer's disease (Haass 1996). Presenilins interact with a minor portion of β-APP molecules, preferentially with the immature N-glycosylated β-APP but not with the (N+O)-glycosylated protein (Weidemann et al. 1997). They may aid in maturation and/or trafficking of β-APP. Mutations in presenilin which cause Alzheimer's disease may induce conformational changes that disturb proper interaction with β-APP and favour fragmentation to $A\beta_{42}$. Production of $A\beta_{42}$ in the ER may thus be the result of a failed quality control, raising the hypothesis that ER-resident β- and γ-secretases may perform initial steps in the degradation of aberrant membrane proteins.

Signal peptide peptidase: release of functional peptides

Signal peptide peptidase (SPPase) has been defined as a proteolytic activity in the ER membrane according to the observation that signal peptides derived from secretory and membrane proteins are cleaved within the transmembrane segment (Lyko et al. 1995). Findings that signal peptide fragments can perform regulatory and other functions in a cell raised the hypothesis that SPPase may play a central role in cellular regulation similar to S2P (Martoglio and Dobberstein 1998).

Signal sequences are essential for targeting secretory proteins and many membrane proteins to the ER membrane and mediate the entry into the secretory pathway (Walter and Johnson 1994). For translocation across the ER membrane, nascent polypeptides enter the protein-conducting channels in a looplike conformation with the N terminus of the signal sequence remaining in the cytosol and the C-terminal portion being translocated (Rapoport et al. 1996). During translocation, signal sequences are usually cleaved off from the precursor protein by signal peptidase (SPase) (Fig. 1c). Cleavage occurs close to the lumenal side of the ER membrane (Dalbey et al. 1997). Liberated signal peptides enter the lipid bilayer and are next cleaved within the hydrophobic membrane-spanning segment by SPPase (Lyko et al. 1995, Martoglio et al. 1997). The resulting N- and C-terminal fragments are released into the cytosol and ER lumen, respectively.

Cleavage by SPPase is sensitive to the immunosuppressive drug cyclosporin A (Klappa et al. 1996). Cyclosporin A usually binds to cellular proteins termed cyclophilins that have proline isomerase activity and are thought to assist protein folding and to modulate the activity of various enzymes (Schreiber and Crabtree 1992). It is therefore conceivable that a cyclophilin in the ER regulates the activity of SPPase.

While SPase is biochemically characterized, SPPase is not identified. The mammalian SPase has been purified as a complex of five subunits with molecular masses of 12, 18, 21, 22/23, and 25 kDa (Evans and Blobel 1986). The 18 and 21 kDa subunits are homologues of the *Escherichia coli* leader peptidase that performs signal sequence cleavage as a single protein. The crystal structure of the latter protein has recently been resolved (Paetzel et al. 1998). The membrane-anchored serine protease contains an exoplasmic domain with the active site close to the membrane. It is thus likely that also the 18 and 21 kDa subunits of the mammalian SPase cleave the signal sequence from the precursor protein. The function of the other SPase subunits is not clear. Further studies will show whether one of them catalyses cleavage of the liberated signal peptide within the transmembrane segment or whether other proteins perform this step.

What could be the role of an SPPase? One function could be to catalyse the first step of signal peptide degradation. It has been shown that synthetic signal peptides enter lipid bilayers and can lyse biological membranes (Hoyt and Girasch 1991, Killian et al. 1990). A mechanism is therefore required to efficiently remove signal peptides from the ER membrane because otherwise their accumulation would severely affect the permeation barrier. Cleavage of liberated signal peptides within the transmembrane segment would facilitate their release from the membrane. A motif for cleavage by SPPase may rely in the helix-break-helix structure that is present in the hydro-

phobic transmembrane segment of signal sequences but usually not in membrane-spanning segments of membrane proteins (van Klompenburg and de Kruijff 1998).

Recent studies revealed that peptides derived from signal sequences have a role in the regulation of cellular functions including virus–host interactions (Martoglio and Dobberstein 1998). In a cell-free system using ER-derived microsomes it has been shown that N-terminal signal peptide fragments of the hormone preprolactin and the HIV-1 envelope protein gp160 are released into the cytosol where they efficiently bind to calmodulin in a calcium-dependent manner (Martoglio et al. 1997). Both signal sequences have properties in their N-terminal extensions that favour binding to calmodulin. Studies in living B cells have revealed a specific association of signal peptide fragments derived from HLA-A, -B, and -C MHC class I molecules with HLA-E MHC class I molecules (Braud et al. 1998). The HLA-E molecules present the signal peptide fragment at the cell surface to inhibitory receptors of natural killer cells. It is thought that the killer cells thereby gauge indirectly via the presented signal sequence fragments the overall level of HLA class I molecules expressed on B cells which is an indicator of the health status of the cell (Long 1998). These findings suggest that the presumably regulated production of signal peptide fragment by SPPase contributes to cellular regulation.

Concluding remarks

Proteases can generate hormonelike signals via a number of mechanisms. Cleavage of transmembrane segments is a unique way in which proteases can liberate active peptides and modulate cell functions. The two-step proteolytic process that includes a transmembrane segment protease seems to be regulated as it is the case for almost all biological systems requiring a series of proteolytic steps. Many questions remain open. What are the sequences and physical properties of transmembrane segment proteases? Do several types of these proteases exist in the ER and elsewhere in the cell? What is the mechanism by which the proteases cleave transmembrane segments? Knowledge about transmembrane segment proteases and the cellular processes they are involved may provide novel targets for therapeutics in treating Alzheimer's disease, cholesterol-induced coronary heart disease, and virus infections.

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